JTP-103237, a monoacylglycerol acyltransferase inhibitor, prevents fatty liver and suppresses both triglyceride synthesis and de novo lipogenesis

AUTHOR(S):

Okuma, Chihiro; Ohta, Takeshi; Tadaki, Hironobu; Ishigure, Tatsuya; Sakata, Shohei; Taniuchi, Hideyuki; Sano, Ryuhei; ... Kume, Shinichi; Nishiu, Jun; Kakutani, Makoto

CITATION:

Okuma, Chihiro ...[et al]. JTP-103237, a monoacylglycerol acyltransferase inhibitor, prevents fatty liver and suppresses both triglyceride synthesis and de novo lipogenesis. Journal of Pharmacological Sciences 2015, 128: 150-157

ISSUE DATE:

2015-04-09

URL:

http://hdl.handle.net/2433/218471

Journal of Pharmacological Sciences 128 (2015) 150–157

HOSTED BY

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

JTP-103237, a monoacylglycerol acyltransferase inhibitor, prevents fatty liver and suppresses both triglyceride synthesis and de novo lipogenesis

Chihiro Okuma [a, *], Takeshi Ohta [a], Hironobu Tadaki [a], Tatsuya Ishigure [a], Shohei Sakata [a], Hideyuki Taniuchi [a], Ryuhei Sano [a], Hiromi Hamada [a], Shinichi Kume [b], Jun Nishiu [a], Makoto Kakutani [a]

[a] *Biological/Pharmacological Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-1 Murasaki-cho, Takatsuki, Osaka 569-1125, Japan*
[b] *Laboratory of Animal Physiology and Functional Anatomy, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan*

ARTICLE INFO

Article history:
Received 9 April 2015
Received in revised form
12 June 2015
Accepted 23 June 2015
Available online 2 July 2015

Keywords:
Monoacylglycerol acyltransferase
Nonalcoholic fatty liver disease
de novo lipogenesis
JTP-103237

ABSTRACT

Aim: Monoacylglycerol acyltransferases (MGATs) are known to play important roles in intestinal TG absorption. In contrast, the role of MGATs in the liver is still unclear. We investigated the effects of JTP-103237, a novel MGAT inhibitor, on hepatic MGAT activity and hepatic lipid metabolism.
Results: JTP-103237 reduced hepatic triglyceride content and hepatic MGAT activity in a high sucrose very low fat (HSVLF) diet induced fatty liver model. Interestingly, JTP-103237 suppressed not only triglyceride (TG) and diacylglycerol (DG) synthesis, but also fatty acid (FA) synthesis (de novo lipogenesis) in this model. JTP-103237 also suppressed lipogenesis-related gene expression, such as sterol regulatory element-binding protein 1-c. Moreover, JTP-103237 decreased plasma glucose levels and total cholesterol and reduced the accumulation of epididymal fats in HSVLF diet fed mice.
Conclusion: In the present study, JTP-103237 prevented carbohydrate-induced fatty liver and suppressed both TG synthesis and de novo lipogenesis, suggesting MGAT inhibitor may prevent carbohydrate-induced metabolic disorders, including NAFLD, obesity and diabetes.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver dysfunction worldwide (1, 2). NAFLD is a condition defined by excessive fat accumulation in the form of triglycerides (TG) in the liver, namely fatty liver. The exact cause of NAFLD is still unknown. However, both obesity and insulin resistance likely have a strong influence on the disease process. Understandably, with the growing epidemic of obesity and diabetes, the prevalence and impact of NAFLD continues to increase (3).

In addition, NAFLD is considered to be associated not only with obese and diabetes onset, but also with cardiovascular disease (4). Inevitably, the economic burden associated with NAFLD will increase; however, effective treatments are not yet available, and hence the need for pharmacological treatments is fully justified.

Fatty liver is considered to result from an imbalance of lipid metabolism (5). Thus, ameliorating hepatic lipid metabolism appears to be a potential strategy for the treatment of NAFLD and other related diseases.

There are two major pathways, the glycerol-3-phosphate (G-3-P) pathway and the monoacylglycerol (MG) pathway, for TG synthesis (6). The G-3-P pathway is a de novo pathway present in most tissues. The MGAT pathway plays a predominant role on dietary fat absorption.

Monoacylglycerol acyltransferases (MGATs) catalyze the first step of TG synthesis. The formation of diacylglycerol (DG) from MG and fatty acyl CoA facilitated by MGAT is considered the

* Corresponding author.
E-mail address: Chihiro.okuma@jt.com (C. Okuma).
Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2015.06.007

rate-limiting step of triacylglycerol synthesis in this pathway (7, 8).

The relevance of MGAT activity to hepatic TG metabolism is still unclear. However, liver MGAT activity reportedly increases in diabetic rats (9) and this pathway is active in neonatal rats (10). In addition, there is evidence that MGAT2 and MGAT3 were overexpressed in patients with NAFLD (11).

In our previous report, we reported our discovery of a novel MGAT2 inhibitor, JTP-103237, which is a 7-(4,6-Di-tert-butyl-pyrimidin-2-yl)-3-(4-trifluoromethoxy-phenyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrazine derivative (12). JTP-103237 selectively inhibited MGAT2 but not DGAT2 and prevented high fat diet induced obesity through the inhibition of intestinal MGAT2. In addition, JTP-103237 reduced hepatic triglyceride content in high fat diet induced obese mice. However, since JTP-103237 reduced food intake and body weight through the inhibition of intestinal MGAT, the contribution of liver MGAT activity on hepatic triglyceride reduction requires further investigation.

In this study, we investigated whether liver MGAT activity is relevant to hepatic lipid metabolism using JTP-103237.

2. Materials and methods

2.1. Chemicals and reagents

JTP-103237 was synthesized in the Central Pharmaceutical Research Institute within Japan Tobacco Inc. (Osaka, Japan). [1-^{14}C] oleoyl-coenzyme A was purchased from Amersham biosciences. [1-^{14}C] oleic acid, [1-^{14}C] palmitic acid and [1-^{14}C] acetate were purchased from PerkinElmer Japan. D-[U-^{14}C]-glucose was purchased from GE Healthcare. All other chemicals were standard reagent grade.

2.2. Animals and diets

Male C57BL/6J mice were purchased from Charles River Laboratories (Yokohama, Japan). Mice were maintained with free access to water and either a normal chow diet (CRF-1, Charles River Japan) or a high sucrose very low fat (HSVLF) diet (D08030601, Research Diets Inc.) The caloric contributions (% fat: % carbohydrate: % protein; kcal/g) in HSVLF diets as indicated by the manufacturer are 2.6: 76.7: 20.7; 3.53 kcal/g, respectively. Animals were housed under specific pathogen-free conditions in a room controlled for temperature at 23 ± 3 °C and humidity of 55 ± 15% in 12-h light/dark cycles (lights on from 8:00 AM to 8:00 PM). All procedures were conducted according to guidelines from Japan Tobacco's Animal Care Committee.

2.3. Measurement of blood chemistry and hepatic triglyceride content

Blood samples were collected from the orbital vein in fed state and plasma glucose, triglyceride and total cholesterol levels were measured by an enzymatic method (glucose: LiquiTech glucose HK test Roche Diagnostics, Switzerland), triglyceride: Determiner L TGII (Kyowa Medex Co, Tokyo, Japan), total cholesterol: Determiner L TCII (Kyowa Medex Co, Tokyo, Japan). Plasma insulin levels were measured by an ELISA (Rat insulin assay kit (Morinaga Institute of Biological Science, Tokyo, Japan). Liver was collected from mice in fed state and a portion of the liver that was approximately 100 mg, 0.5 ml of methanol, and zirconia beads were added to tubes. The portion of liver was homogenized using a mixer mill (MM300 Retch) (25 Hz, 10 min). To the homogenized solution, 1 ml of chloroform was added and mixed thoroughly. The mixture was then centrifuged (10,000 g, 5 min, 4C°) and the resulting supernatant collected. Solvents contained in 0.5 ml of the supernatant were dried under a stream of nitrogen gas. To the residue, 0.5 ml of 2-propanol was added to reconstitute the residue. The TG concentration of the 2-propanol solution was determined by an enzymatic method.

2.4. Evaluation on liver MGAT activity

Liver S9 fractions were isolated through centrifugation as previously described (12). The MGAT assay was performed at room temperature in the presence of 100 mM Tris–HCl (pH 7.5), 250 mM Sucrose, 5 mM $MgCl_2$, 0.05% BSA (Sigma–Aldrich, St. Louis, MO), 0.05 mM 2-oleoyl glycerol and 25 μM [1-^{14}C] oleoyl-CoA. The radioactivity of synthesized [1-^{14}C] diacylglycerol was separated using thin layer chromatography (TLC) and analyzed as MGAT activity.

2.5. Evaluation of hepatic lipid synthesis

Livers were collected in fed state from mice and a portion of the liver that was approximately 100 mg was added to reaction medium (DMEM low glucose containing 500 μM [1-^{14}C] acetate (29.0 μCi/ml)). After 90 min, the reaction was stopped by fast cooling. Lipids were extracted from portions of the liver, and separated via TLC using a hexane:diethylether:acetic acid (80:30:2) solvent system. The signal intensity of the TG and DG fraction on a TLC plate was measured using an FLA-7000 imaging system. The radioactivity equivalent in each sample was calculated using a correction factor obtained from the standard sample and the ratio of lipid synthesis per 90 min per gram of liver was also calculated.

The lipid extract was saponified with 1.5 N NaOH (90 min, 4C°). After saponification, lipids were re-extracted and separated via TLC using the same process described above. The signal intensity of the fatty acid fraction on a TLC plate was measured and the radioactivity equivalent in each sample was calculated in the same manner as previously mentioned. The rate of fatty acid synthesis (de novo lipogenesis) was calculated as nmol of radioactivity incorporated into fatty acid fractions per 90 min per gram of liver.

2.6. Evaluation of hepatic lipid components after administration of fatty acids

10-week old male C57BL/6J mice were acclimatized to an HSVLF diet for 5 weeks. JTP-103237 was administered orally to mice in fed state at a dose of 100 mg/kg. After 60 min, 30 μM/kg of oleic acid containing 135 μCi/kg of [1-^{14}C] oleic acid was intraperitoneally administered. After 15 min, mice were sacrificed and a portion of the liver that was approximately 350 mg was collected. Lipids were extracted and separated via TLC. The signal intensity of TG, DG fatty acid and phospholipid fractions on a TLC plate were measured using an FLA-7000 imaging system as described above. The ratio of each lipid component to total lipids was calculated.

2.7. mRNA quantification with real-time quantitative PCR

Total RNA was extracted from the liver. RNA was transcribed into cDNA using M-MLV reverse transcriptase and random primers (Invitrogen, Carlsbad, CA). The reaction mixture was incubated for 10 min at 25 °C, 1 h at 37 °C, and 5 min at 95 °C. Real-time PCR quantification was performed in a 50-μL reaction mixture with an automated sequence detector combined with the ABI Prism 7700 Sequence Detection System software (Applied Biosystems, Foster City, CA). The reaction mixture contained 50 ng of synthesized cDNA, 3.5 mM $MgCl_2$, 0.3 μM primers, 0.1 μM probes, and

1.25 units of Ampli Taq Gold®. Cycle parameters were 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The expression of mRNA levels were normalized using 18s rRNA levels. The following primers and FAM-conjugated probes were designed using Primer Express software (Applied Biosystems): sterol regulatory element-binding protein 1-c (SREBP-1c) (forward, ATCGGCGCGGAAGCTGTCGGGGTAGCGTC; reverse, TGAGCTGGAGC ATGTCTTCAA; probe, ACCACGGAGCCATGGATTGCACATT), stearoyl-CoA desaturase 1 (SCD-1) (forward, TCTCCAGTTCTTACACGACC ACC; reverse, GGACGGATGTCTTCTTCCAGGT; probe, CCTCCGGAAAT GAACGAGAGAAGGTGAAG), fatty acid synthase (FAS) (forward, GGTCTATGCCACGATTCTGAAT; reverse, GGAATGTTACACCTTGCTC CTT; probe, CACCAATACAGATGGCAG), Acetyl-CoA carboxylase (ACC) (forward, TGGATGATGGTCTGAAGGCAG; reverse, CCTCTGAG GCCTTGATCATCAC; probe, TGAGGAAGTTGGCTATCC), mitochondrial glycerol 3-phosphate acyltransferase (mtGPAT) (forward, GCAATGGCGTACTTCATGTGTT; reverse, CATGGAAGCCATCATAGCTT GCAGCATC; probe, GCACCTCTTATTCAGGACTGCA), diacyl glycerolacyltransferase 2 (DGAT2) (forward, ATCCTCATGTACACCTTCTG CACAG; reverse, ACCTGGCTGGCATTTGACTGGAACA; probe, ATCTC CTGCCACCTTTCTTGG), and 18s rRNA (purchased from Applied Biosystems).

2.8. Glucose utilization in fat tissue

Small portions (approximately 200 mg) of epididymal and mesenteric adipose tissues were incubated in Hank's balanced salt solution (pH7.4) containing D-[U-^{14}C]-glucose in the absence or presence of insulin (1 or 10 nmol/l) at 37 °C for 2 h. After stopping the reaction through the addition of 0.05 mol/l H_2SO_4, the $^{14}CO_2$ produced was trapped in filter paper. Radioactivity in the filter paper was measured using a liquid scintillation counter (TRI-CARB 2500TR, Packard BioScience, Waltham, MA, USA).

2.9. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). All statistical analysis was performed with statistical software Statlight 2000 (Yukms Corp., Tokyo, Japan). A Student's t-test was performed provided that homogeneity was confirmed by an F test. In the case that homogeneity was not confirmed by an F test, a Welch's test was performed. A Dunnett's multiple comparison test was performed in the multiple-group study provided that homogeneity was confirmed by a Bartlett's homoscedasticity test. In the case that homogeneity was not confirmed by a Bartlett's homoscedasticity test, a Steel's multiple comparison test was performed. A Pearson correlation test was used to analyze the correlation between hepatic triglyceride levels and MGAT activity. Differences were considered significant if P was <0.05 (2-sided).

3. Results

3.1. Effects on hepatic triglycerides and liver MGAT activity in HSVLF diet fed mice

To investigate whether JTP-103237 decreases liver lipid synthesis by inhibiting liver MGAT activity, hepatic triglyceride content in HSVLF diet fed mice was evaluated.

In HSVF fed mice, hepatic triglyceride levels and MGAT activity increased as compared to those in normal diet fed mice. The level of liver MGAT activity in this study was 6.3*10^{-5} nmol/mg/min

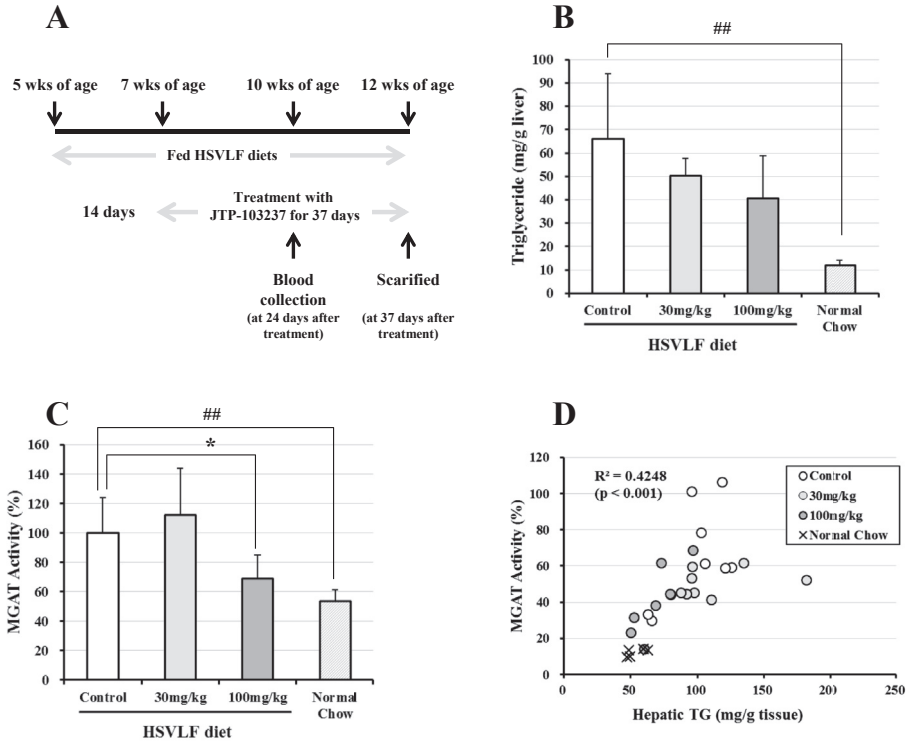

Fig. 1. Hepatic triglycerides and MGAT activity after chronic treatment with JTP-103237. 5-week old mice were fed HSVLF diet for two weeks. Subsequently, JTP-103237 was administered as a food admixture (the final dosages were 33 mg/kg/day in the 30 mg/kg group and 111 mg/kg/day in the 100 mg/kg group). After 37 days, mice were sacrificed in fed state and livers were collected. Hepatic triglyceride content and MGAT activity were measured as described in the materials and methods section. The scheme of the treatment (A), Hepatic triglycerides (B), MGAT activity (C) and the relationship between both (D) are shown. Data represent the mean ± S.D (n = 8/group in the HSVLF diet fed groups, n = 6 in the normal chow group). *P < 0.05, **P < 0.01 vs. control (Dunnett's), ##P < 0.05 vs. normal chow fed group (Student's t-test). In the figure (C), **P < 0.01 by Pearson tests.

Table 1
Biochemical parameters in JTP-103237 treated mice fed an HSVLF diet. 5-week old mice were fed HSVLF diet for two weeks. Subsequently, JTP-103237 was administered as a food admixture (the final dosages were 33 mg/kg/day in the 30 mg/kg/day group and 111 mg/kg/day in the 100 mg/kg/day group). On Day 24, blood was collected from the orbital vein in fed state and plasma glucose, insulin, triglyceride and total cholesterol levels were measured. The values for body weight and cumulative food intake were those obtained on Day 37. On day 37, epididymal fat was collected and wet weight was measured. Data represent the mean ± S.D (n = 8/group in the HSVLF diet fed groups, n = 6 in the normal chow group).

Chow	Normal	HSVLF		
Treatment	None	None	JTP-103237 30 mg/kg/day	JTP-103237 100 mg/kg/day
Body weight (g)	27.5 ± 0.2	27.2 ± 1.8	26.9 ± 0.7	26.7 ± 1.7
Cumulative food intake (g)	122 ± 4	113 ± 7[d]	116 ± 4	116 ± 8
Epididymal fat weight (g)	0.54 ± 0.06	0.65 ± 0.15	0.60 ± 0.10	0.49 ± 0.11[a]
Glucose (mg/dl)	186 ± 14	215 ± 41	197 ± 26	179 ± 18[a]
Insulin (mg/dl)	2.0 ± 2.0	3.5 ± 2.1	1.5 ± 1.1[c]	1.7 ± 0.8
Triglycerides (mg/dl)	144 ± 23	74 ± 26[d]	59 ± 13	58 ± 21
Total cholesterol (mg/dl)	103 ± 2	125 ± 16[e]	123 ± 10	96±9[b]

[a] $P < 0.05$.
[b] $P < 0.01$ vs. control (Dunnett's test).
[c] $P < 0.05$ vs. control (a Steel's multiple comparison test).
[d] $P < 0.05$ vs. normal chow fed group (Student's t-test).
[e] $P < 0.01$ vs. normal chow fed group (Student's t-test).

(average) in HSVLF diet fed mice. Although, the values were not statistically significant, JTP-103237 tended to decrease hepatic triglyceride in particular 100 mg/kg/day. In 30 mg/kg/day treated group, these changes were not clear. JTP-103237 decreased MGAT activity at a dose of 100 mg/kg/day (Fig. 1B–C). In addition, there was a statistically significant correlation between hepatic TG and MGAT activity (Fig. 1D). Hepatic triglycerides were evaluated again in additional study at 100 mg/kg/day of JTP-103237 for 21 days. The results showed that JTP-103237 significantly decreased hepatic triglyceride (45.0 ± 5.5 mg/g liver in vehicle group and 27.1 ± 7.3 mg/g liver, respectively. $P < 0.01$ in Student's t-test).

3.2. Effects on body weight, food intake, fat weight and biochemical parameters in HSVLF diet fed mice

As shown in Table 1, JTP-103237 did not alter body weight and cumulative food intake in HSVLF diet fed mice. On the other hand, JTP-103237 decreased fat weight, plasma glucose and total cholesterol levels at a dose of 100 mg/kg/day. In addition, plasma insulin levels were tended to be decreased in JTP-103237 treated groups and it was statistically significant at 30 mg/kg/day. Although, these values were not statistically significant, JTP-103237 tended to decrease plasma triglyceride levels.

3.3. Effect on hepatic lipid synthesis in HSVLF diet fed mice

Subsequently, the effect of chronic treatment of JTP-103237 on hepatic lipid synthesis in isolated liver tissues in HSVLF diet fed mice was evaluated. JTP-103237 significantly suppressed TG and DG synthesis from acetates (Fig. 2A). In addition, lipid extracts were saponified through treatment with NaOH to investigate de novo lipogenesis (fatty acid synthesis). As a result, JTP-103237 also significantly decreased de novo lipogenesis (Fig. 2B).

In order to clarify the time sequence of the effects, lipogenesis after a single or subchronic treatment of JTP-103237 was evaluated. JTP-103237 significantly suppressed lipogenesis after 7 days of treatment but did not suppress lipogenesis after a single treatment (Fig. 3).

3.4. Effect on lipogenic gene expression

The mRNA levels of lipogenic genes (SREBP-1c, SCD-1, FAS, ACC, mtGPAT and DGAT2) in the liver in chronic JTP-103237 treated

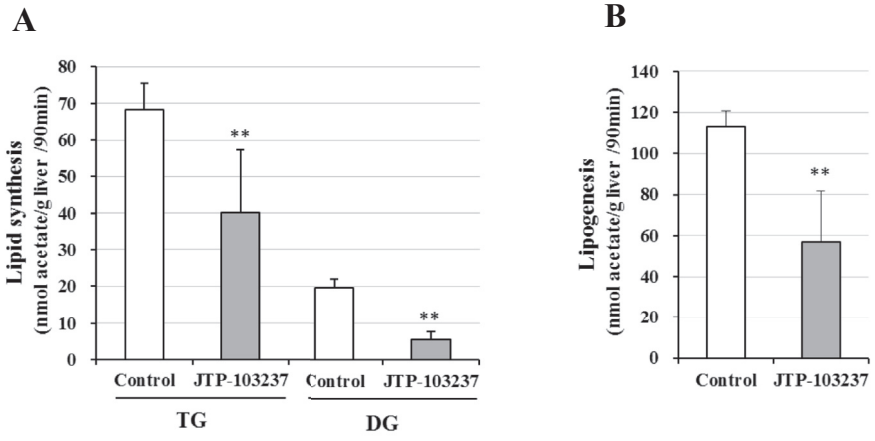

Fig. 2. TG, DG and fatty acid synthesis from acetate in isolated liver tissues after chronic treatment with JTP-103237. (A) 7-week old mice were fed HSVLF diet for 1 week. Subsequently, JTP-103237 was administered as a food admixture (the final dosage was 104 mg/kg/day in the 100 mg/kg group). After 21 days, mice were sacrificed in fed state and livers were collected. The rate of TG and DG synthesis from acetate was evaluated as described in the materials and methods section. (B) Using the sample in (A), fatty acid content after saponification was evaluated as fatty acid synthesis (de novo lipogenesis). Data represent the mean ± S.D. (n = 5/group). *P < 0.05, **P < 0.01 vs. control (Student's t-test), ††P < 0.01 vs. control (Welch's t test).

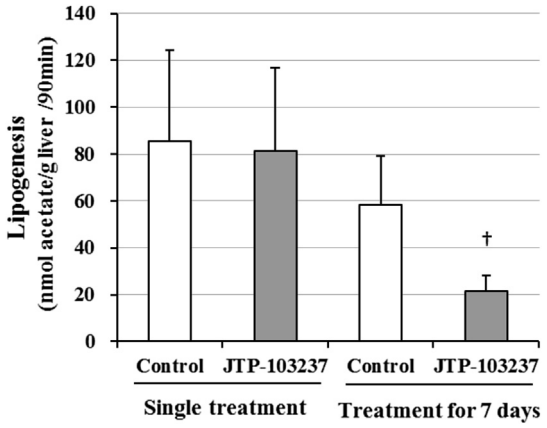

Fig. 3. Lipogenesis after a single or 7 days of treatment with JTP-103237. 7-week old mice were fed HSVLF diet for 1 week. In the single dosing experiment, JTP-103237 was administered orally at a dose of 100 mg/kg. After 60 min, mice were sacrificed in fed state and livers were collected. In the study with 7 days dosing, JTP-103237 was administered as a food admixture (the final dosage was 104 mg/kg/day in the 100 mg/kg group). After 7 days, mice were sacrificed in fed state and livers were collected. The rate of fatty acid synthesis (de novo lipogenesis) from acetate was evaluated as described in the materials and methods section for both experiments. Data represent the mean ± S.D. (n = 5/group). *P < 0.05 vs. control (Student's t-test).

HSVLF diet fed mice were measured using real-time PCR. SREBP-1c, SCD-1 and DGAT2 genes expression was significantly decreased in JTP-103237 treated mice as compared to control (Fig. 4). FAS, ACC and mtGPAT genes expression did not yield statistically significant differences due to the large variability observed, but tended to decrease as compared to control.

3.5. Effects on hepatic lipid components after administration of fatty acids

The amount of lipid components after a single treatment of JTP-103237 was subsequently analyzed using ^{14}C labeled oleic acid. The differences were not statistically significant; however, JTP-103237 tended to decrease the ratio of TG (product of MGAT) to total labeled lipids (P = 0.06). In addition, JTP-103237 significantly increased the ratio of fatty acids (substrate of MGAT) to total labeled lipids (Table 2). However, since separating the bands for MG was difficult, the amount of MG could not be evaluated in this experiment. The ratio of DG did not change in this study, which was likely due to the difficulty in determining DG levels, since DG is an intermediate product of triacylglycerol synthesis.

Table 2
Lipid composition after administration of 14C oleic acid in JTP-103237 treated mice fed an HSVLF diet. 10-week old male C57BL/6J mice were acclimatized to an HSVLF diet for 5 weeks. JTP-103237 was administered orally to mice in fed state at a dose of 100 mg/kg. After 60 min, 30 μM/kg of oleic acid containing 135 μCi/kg of [1-^{14}C] oleic acid was intraperitoneally administered. After 15 min, mice were sacrificed and hepatic lipids were extracted. The signal intensity of TG, DG, fatty acid and phospholipid fractions on a TLC plate and the ratio of each lipid component to total lipids were calculated. Data represent the mean ± S.D (n = 6/group).

	TG (%)	DG (%)	Fatty acid (%)	Phospholipid (%)
Control	72.1 ± 2.2	17.7 ± 2.9	5.0 ± 0.5	5.0 ± 0.9
JTP-103237	69.8 ± 1.3	20.0 ± 1.6	5.5 ± 0.3[a]	4.9 ± 0.9

[a] P < 0.05 vs. control (Student's t-test).

3.6. Effects on insulin sensitivity in isolated fat tissues

To investigate the effects of JTP-103237 in other tissues, adipose glucose oxidation in HSVLF diet fed mice was evaluated. Glucose oxidation capability increased with insulin in both epididymal and mesenteric fat tissue. As shown in Fig. 5, it was not statistically significant, but glucose oxidation in JTP-103237 treated adipose tissues tended to increase and these effects with insulin were clearer than those without insulin. The P value in mesenteric fat tissue with 10 nM insulin was 0.0977.

4. Discussion

MGATs are known to play important roles in intestinal TG absorption. In addition to intestinal MGAT activity, there are a few reports describing liver MGAT activity (9–11). Regarding subtype, very low expression levels of both MGAT1 and MGAT2 were reportedly detected in mouse liver (13, 14) Although it was shown that systemic Mogat2 deficient mice are resistant to high fat diet induced obesity and fatty livers (15), the role of hepatic MGAT has been still unclear. On the other hand, in a recent report, it was indicated MGAT2 in extraintestinal tissues may contribute to the regulation of energy metabolism (16, 17). For example, intestine-specific expression of Mogat2 in systemic Mogat2 deficient mice partly restored the metabolic changes. In addition, MGAT1 antisense oligonucleotides treatment, reportedly, improved fatty liver and hepatic insulin sensitivity (18).

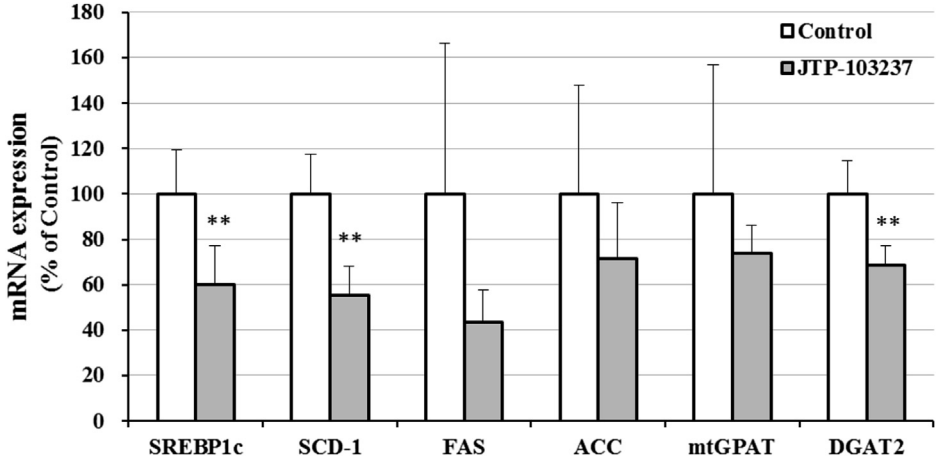

Fig. 4. Effects of JTP-103237 on lipogenic gene expressions. 6-week old mice were fed HSVLF diet for 1 week. Subsequently, JTP-103237 was administered as a food admixture (the final dosage was 102 mg/kg/day in the 100 mg/kg group). After 29 days, mice were sacrificed in fed state and livers were collected. Total RNA was extracted from the livers and lipogenic gene expression was measured using quantitative real-time PCR. Data represent the mean ± S.D. (n = 6/group). **P < 0.01 vs. control (Student's t-test).

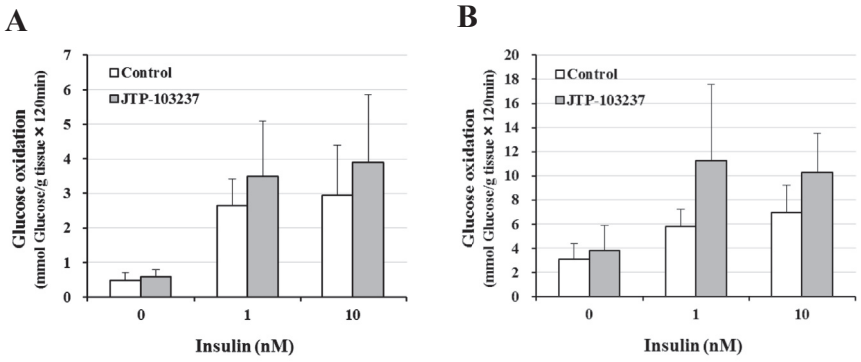

Fig. 5. Glucose utilization in epididymal fat tissue (A) and mesenteric fat tissue (B) after chronic treatment with JTP-103237. 7-week old mice were fed HSVLF diet for 1 week. Subsequently, JTP-103237 was administered as a food admixture (the final dosage was 104 mg/kg/day in the 100 mg/kg group). After 44days, mice were sacrificed in fed state and a small portion of epididymal and mesenteric adipose tissues were collected. Glucose utilization was measured in the absence or presence of insulin (1 or 10 nmol/l). Data represent the mean ± S.D. (n = 5/group).

Therefore, we hypothesized that extraintestinal MGAT, in particular hepatic MGAT, might be involved in hepatic lipid metabolism.

In the present study, we investigated the effects of MGAT inhibition on hepatic lipid metabolism using JTP-103237, a novel MGAT inhibitor. As shown in previous report, JTP-103237 inhibited MGAT2 activity, but did not inhibit DGAT2 activity, which belongs to the same family as MGAT (12). In addition, the IC50 value for MGAT3 was approximately 300 times higher than that of MGAT2. However, we have not evaluated the effect of JTP-103237 on MGAT1 activity since we could not obtain the membrane fraction which exhibited sufficient MGAT1 activity. Therefore, there is a probability that JTP-103237 inhibits not only MGAT2 but MGAT1 in the present study.

In order to focus on liver MGAT activity, we used HSVLF diets in the present study, which induced fatty livers due to high sucrose content (19). Since the caloric contribution of fat in HSVLF diet was only 2.6%, the effects by intestinal MGAT inhibition were considered small. Indeed, JTP-103237 did not reduce body weight and food intake in HSVLF diet fed mice in the present study in contrast to previous report using high fat diet fed mice (12).

Although the values were not statistically significant, JTP-103237 tended to decrease hepatic triglyceride in this study. In addition, in the reproducibility study, JTP-103237 significantly decreased hepatic triglycerides at 100 mg/kg/day. Taken together, it is considered JTP-103237 decreased hepatic triglycerides in HSVLF diet fed mice. Moreover, the relationship between hepatic TG content and MGAT activity was significantly correlated, suggesting that decreases in carbohydrate-induced fatty liver might be due to hepatic MGAT inhibition.

We next investigated lipid synthesis in the liver in HSVLF diet fed mice after chronic treatment with JTP-103237. JTP-103237 decreased TG and DG synthesis after chronic treatment. These results were within expectation; however, surprisingly, JTP-103237 decreased fatty acid synthesis from acetate (de novo lipogenesis).

MGAT catalyzes the synthesis of DG from MG and is the key enzyme for triglyceride synthesis. Understandably, this is not relevant to fatty acid synthesis. We suspected non-specific inhibitory effects on enzymes related to fatty acid synthesis and we, thus, investigated the effects of JTP-103237 on lipogenesis after a single treatment. Results demonstrated that JTP-103237 did not decrease de novo lipogenesis after a single treatment but decreased lipogenesis after 7 days of treatment. From these findings, the decrease in de novo lipogenesis by JTP-103237 is considered to be not due to an acute effect of hepatic MGAT inhibition.

Next, to understand how JTP-103237 decreased carbohydrate-induced fatty liver, we analyzed genes involved in lipogenesis. Interestingly, the expression of SREBP-1c, SCD1 and DGAT2 genes was significantly decreased with chronic treatment of JTP-103237. Preliminarily, the expression of MGAT1 and MGAT2 gene were also evaluated. The expression of MGAT1 was not changed and that of MGAT2 tended to be decreased ($P = 0.078$, Student's t-test). However, since the copy number of these mRNA was low in comparison with other mRNAs, the data have not been shown. In addition, the expression of LXR and other SREBPs were not evaluated but these are subjects for future investigation.

SREBP-1c is a key regulator of fatty livers (20) and the overexpression of SREBP-1c in the liver of transgenic mice is known to manifest as severe fatty livers (21). SREBP-1c is predominantly involved in the regulation of insulin-responsive genes, which control lipogenesis (22). In particular, SCD1, which is regulated via SREBP-1c, plays an important role in fatty liver and insulin resistance (23). Indeed, liver-specific SCD1 deficiency completely blocked carbohydrate-induced lipogenesis (24).

Fatty acids, which are substrates of MGAT, are known to inhibit SREBP-1c expression by antagonizing activation of the liver X receptor (LXR) signaling (25, 26). We hypothesized that fatty acids accumulated by inhibiting hepatic MGAT might inhibit LXR activation and consequent SREBP-1c expression. Next, to investigate this hypothesis, we evaluated the effects of a single treatment of JTP-103237 on hepatic lipid components after administration of fatty acids. Results demonstrated that JTP-103237 significantly increased the ratio of fatty acids, and tended to decrease the ratio of TG in liver. These results suggested the possibility that hepatic MGAT inhibition may decrease not only TG synthesis, but also de novo lipogenesis via suppression of LXR/SREBP-1c signaling pathway by increasing fatty acid. As described above, JTP-103237 decreased DGAT2 gene expression. It is known that DGAT2, which is strongly involved in fatty liver (27), was not regulated by SREBP-1c but LXR (28, 29). This result also indicated that JTP-103237 modulates the LXR/SREBP-1c signaling pathway. Our postulated mechanisms on the decrease in hepatic lipid synthesis by JTP-103237 are shown in Fig. 6.

The World Gastroenterology Organization stated that NAFLD and nonalcoholic steatohepatitis (NASH) represent a major global public health problem that is pandemic (2). However, there are no specific drugs for the treatment of these diseases. Recently,

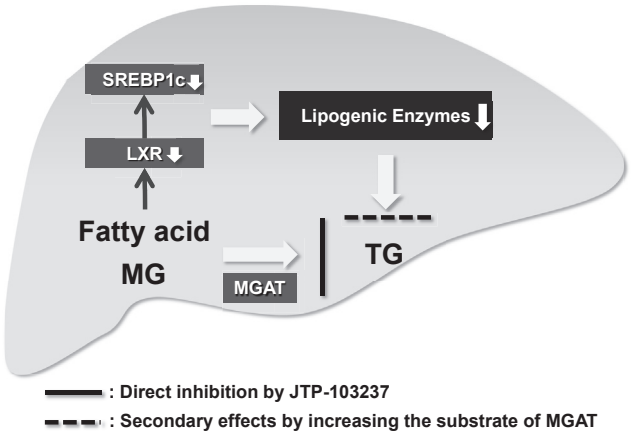

Fig. 6. Postulated mechanisms on decrease in hepatic lipid synthesis by an MGAT inhibitor. TG synthesis via the MGAT pathway may be suppressed through the direct inhibition of MGAT. As a result of MGAT inhibition, fatty acids, which are substrates of MGAT, may increase and it suppresses SREBP-1c and related gene expression via antagonizing LXR signaling (secondary effects through the inhibition of MGAT). Hepatic TG lipid synthesis would be suppressed by the MGAT inhibitor via direct and indirect (secondary) effects.

the inhibition of hepatic de novo lipogenesis has been considered to be a potential therapeutic strategy for NAFLD (30). The contribution of de novo lipogenesis to total hepatic triglyceride production in normal subjects is small. However, the contribution of de novo lipogenesis to total hepatic triglyceride production in patients with NAFLD is much higher (31). In the present study, JTP-103237 prevented carbohydrate-induced fatty liver and suppressed both TG synthesis and de novo lipogenesis, suggesting that MGAT inhibition might be potential target for treatment of NAFLD and NASH.

JTP-103237 decreased plasma glucose levels and total cholesterol, reduced the accumulation of epididymal fats and tended to decrease plasma insulin levels. Since it had been observed the reduction of fat tissue and plasma insulin levels, the insulin sensitivity in adipose tissue was investigated. Although the changes were not statistically significant due to large variation, JTP-103237 tended to increase adipose tissue glucose oxidation with insulin in HSVLF diet fed mice. In mesenteric fat tissue, the percentages for control group with insulin were 193% and 148% (with 1 and 10 nM insulin, respectively) whereas that without insulin was 125%. There is room for further investigation regarding the role of MGAT in extraintestinal tissue including muscle, but it may be probable that MGAT involves in systemic energy metabolism and insulin sensitivity.

From these findings, JTP-103237 is expected to display beneficial effects in the treatment of carbohydrate induced metabolic disorders, including NAFLD, adiposity and diabetes. In our previous report, JTP-103237 also prevented fat induced obesity, fatty liver formation and glucose intolerance (12). Taken together, MGAT inhibitor would be effective in metabolic diseases, regardless of whether these diseases are caused by excessive fat intake or carbohydrate intake.

Conflicts of interest

All the authors indicated no potential conflicts of interest.

References

(1) Attar BM, Van Thiel DH. Current concepts and management approaches in nonalcoholic fatty liver disease. ScientificWorldJournal. 2013;2013:481893.
(2) Review T, LaBrecque DR, Abbas Z, Anania F, Ferenci P, Khan AG, et al. World Gastroenterology Organisation global guidelines: nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. J Clin Gastroenterol. 2014;48:467–473.
(3) Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34:274–285.
(4) Seage 3rd GR, Horsburgh Jr CR, Hardy AM, Mayer KH, Barry MA, Groopman JE, et al. Increased suppressor T cells in probable transmitters of human immunodeficiency virus infection. Am J Public Health. 1989;79:1638–1642.
(5) Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). Prog Lipid Res. 2009;48:1–26.
(6) Shi Y, Cheng D. Beyond triglyceride synthesis: the dynamic functional roles of MGAT and DGAT enzymes in energy metabolism. Am J Physiol Endocrinol Metab. 2009;297:E10–E18.
(7) Yen CL, Farese Jr RV. MGAT2, a monoacylglycerol acyltransferase expressed in the small intestine. J Biol Chem. 2003;278:18532–18537.
(8) Senior JR, Isselbacher KJ. Direct esterification of monoglycerides with palmityl coenzyme A by intestinal epithelial subcellular fractions. J Biol Chem. 1962;237:1454–1459.
(9) Mostafa N, Bhat BG, Coleman RA. Increased hepatic monoacylglycerol acyltransferase activity in streptozotocin-induced diabetes: characterization and comparison with activities from adult and neonatal rat liver. Biochim Biophys Acta. 1993;1169:189–195.
(10) Bhat BG, Bardes ES, Coleman RA. Solubilization and partial purification of neonatally expressed rat hepatic microsomal monoacylglycerol acyltransferase. Arch Biochem Biophys. 1993;300:663–669.
(11) Hall AM, Kou K, Chen Z, Pietka TA, Kumar M, Korenblat KM, et al. Evidence for regulated monoacylglycerol acyltransferase expression and activity in human liver. J Lipid Res. 2012;53:990–999.
(12) Okuma C, Ohta T, Tadaki H, Hamada H, Oda T, Taniuchi H, et al. JTP-103237, a novel monoacylglycerol acyltransferase inhibitor, modulates fat absorption and prevents diet-induced obesity. Eur J Pharmacol. 2015;758:72–81.
(13) Cao J, Lockwood J, Burn P, Shi Y. Cloning and functional characterization of a mouse intestinal acyl-CoA:monoacylglycerol acyltransferase, MGAT2. J Biol Chem. 2003;278:13860–13866.
(14) Yen CL, Stone SJ, Cases S, Zhou P, Farese Jr RV. Identification of a gene encoding MGAT1, a monoacylglycerol acyltransferase. Proc Natl Acad Sci U. S. A.. 2002;99:8512–8517.
(15) Yen CL, Cheong ML, Grueter C, Zhou P, Moriwaki J, Wong JS, et al. Deficiency of the intestinal enzyme acyl CoA:monoacylglycerol acyltransferase-2 protects mice from metabolic disorders induced by high-fat feeding. Nat Med. 2009;15:442–446.
(16) Nelson DW, Gao Y, Yen MI, Yen CL. Intestine-specific deletion of acyl-CoA:monoacylglycerol acyltransferase (MGAT) 2 protects mice from diet-induced obesity and glucose intolerance. J Biol Chem. 2014;289:17338–17349.
(17) Gao Y, Nelson DW, Banh T, Yen MI, Yen CL. Intestine-specific expression of MOGAT2 partially restores metabolic efficiency in Mogat2-deficient mice. J Lipid Res. 2013;54:1644–1652.
(18) Soufi N, Hall AM, Chen Z, Yoshino J, Collier SL, Mathews JC, et al. Inhibiting monoacylglycerol acyltransferase 1 ameliorates hepatic metabolic abnormalities but not inflammation and injury in mice. J Biol Chem. 2014;289:30177–30188.
(19) Miyazaki M, Flowers MT, Sampath H, Chu K, Otzelberger C, Liu X, et al. Hepatic stearoyl-CoA desaturase-1 deficiency protects mice from carbohydrate-induced adiposity and hepatic steatosis. Cell Metab. 2007;6:484–496.
(20) Ahmed MH, Byrne CD. Modulation of sterol regulatory element binding proteins (SREBPs) as potential treatments for non-alcoholic fatty liver disease (NAFLD). Drug Discov Today. 2007;12:740–747.
(21) Matsuda M, Korn BS, Hammer RE, Moon YA, Komuro R, Horton JD, et al. SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. Genes Dev. 2001;15:1206–1216.
(22) Foretz M, Guichard C, Ferre P, Foufelle F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proc Natl Acad Sci U. S. A.. 1999;96:12737–12742.
(23) Flowers MT, Miyazaki M, Liu X, Ntambi JM. Probing the role of stearoyl-CoA desaturase-1 in hepatic insulin resistance. J Clin Invest. 2006;116:1478–1481.
(24) Flowers MT, Keller MP, Choi Y, Lan H, Kendziorski C, Ntambi JM, et al. Liver gene expression analysis reveals endoplasmic reticulum stress and metabolic dysfunction in SCD1-deficient mice fed a very low-fat diet. Physiol Genomics. 2008;33:361–372.
(25) Jump DB, Clarke SD, Thelen A, Liimatta M. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. J Lipid Res. 1994;35:1076–1084.
(26) Ou J, Tu H, Shan B, Luk A, DeBose-Boyd RA, Bashmakov Y, et al. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc Natl Acad Sci U. S. A.. 2001;98:6027–6032.

(27) Wang Z, Yao T, Song Z. Involvement and mechanism of DGAT2 upregulation in the pathogenesis of alcoholic fatty liver disease. J Lipid Res. 2010;51:3158–3165.
(28) Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, et al. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc Natl Acad Sci U. S. A.. 2003;100:12027–12032.
(29) Han CC, Wang JW, Pan ZX, Tang H, Xiang SX, Wang J, et al. Effect of liver X receptor activation on the very low density lipoprotein secretion and messenger ribonucleic acid level of related genes in goose primary hepatocytes. Poult Sci. 2011;90:402–409.
(30) Lambert JE, Ramos-Roman MA, Browning JD, Parks EJ. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. Gastroenterology. 2014;146:726–735.
(31) Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology. 2010;51:679–689.